Observations on the Digestive Ferments.

By R. H. CHITTENDEN, Ph. D.,

PROFESSOR OF PHYSIOLOGICAL CHEMISTRY IN YALE UNIVERSITY.

[FROM THE PHILADELPHIA MEDICAL NEWS, FEBRUARY 16, 1889.]

Observations on the Digestive Ferments.

READ BEFORE THE SECTION ON PÆDIATRICS OF THE NEW YORK ACADEMY OF MEDICINE, JANUARY 23, 1889.

BY R. H. CHITTENDEN, PH. D.,

PROFESSOR OF PHYSIOLOGICAL CHEMISTRY IN YALE UNIVERSITY.

Recent years have witnessed a marked development in our knowledge of the physiology of digestion. A great mass of histological and physiological details has been gradually accumulated by which a clearer insight has been obtained into many of the processes of secretion, digestion and absorption. Chemical science has lent its aid and given us light on the composition and character of the digestive juices, and on many hitherto obscure points in the metamorphism of the various food stuffs, and at the same time taught us to appreciate its value in the study of scientific medicine.

There is, I think, no branch of medicine where a proper appreciation and true understanding of physiological processes is so necessary as in the pathology of digestion. In the words of Dr. Ewald, "digestion is comparable to a complicated clock-work, the derangements of which are readily shown by the movements of the hands, but the causes of which are difficult to discover from the complexity and concealed position of the movement. Therefore, the pathology of digestion requires a well grounded knowledge of the complex processes which affect the transformation of our food into chyle."

Among the more recently acquired facts pertaining to digestion none are of more importance than those which relate to the digestive ferments. In the processes going on in the alimentary canal, by which the nutritive portions of the food are transformed into soluble and diffusible products fitted for the nourishment of the blood and of the tissues, ferments play an all-important part; without the action of the unorganized ferments, the nutrition and life of the organism would be impossible. As you well know, the more important digestive ferments or enzymes are of two kinds, the amylolytic, acting on starchy matter and the proteolytic, acting on the albuminous food stuffs. As examples of the former we have the ptyalin of saliva and the amylopsin of pancreatic juice, as well as the diastatic ferment of the bile; as examples of the latter, the pepsin of the gastric juice and the trypsin of the pancreatic fluid.

The terms fermentation and ferment may be variously defined, but yet after all we cannot add much to the definition current in the fourteenth century, viz.: "A force, which, without becoming weaker itself, can produce great effects in other masses." We know that this is not strictly correct, yet the amount of ferment involved in any given fermentation is, as a rule, so infinitesimally small, so out of proportion to the magnitude of the chemical processes or changes caused by it, and at the same time so continuous in its action, that we may well marvel at its power and wonder at its methods. We feign knowledge, however, and call it a *catalytic* action, a term which clearly exposes our ignorance, but which helps to foster our self-esteem.

The amylolytic and proteolytic ferments are alike in that they act only in the presence of water; that the products of their action contain, as a rule, more oxygen and hydrogen than the original matter, thus implying a hydration process; and that their action is most energetic at the temperature of body. They differ, however, in the medium in which they act; the amylolytic ferments being most energetic in a neutral fluid, wholly inactive in the presence of free acids; the proteolytic ferment pepsin, on the other hand, acting only when in combination with an acid, preferably hydrochloric, while trypsin acts best in an alkaline medium, although also active in a neutral fluid. These what may be called minor points of difference, are essential ones, however, and serve important purposes in the economy. For the ferments are extremely sensitive to the action of foreign matters and the simple changes of reaction from acid to alkaline and vice versa met with in the alimentary tract are sufficient to destroy the different ferments as they are exposed to the changed conditions in their journey onward and doubtless only such escape destruction as are absorbed and ultimately excreted through the kidneys. Thus, as we shall see, the amylolytic ferment secreted by the salivary glands is undoubtedly destroyed by the acid of the gastric juice, the proteolytic and rennet ferments, secreted by the gastric glands are destroyed by the conjoined action of trypsin and the alkaline salts of the pancreatic and intestinal secretions, while the ferments of the pancreatic juice are probably in turn destroyed, at least in great part, by the acids of the large intestines.

Bearing in mind the extreme importance to the economy of these unorganized ferments, it may not be amiss to consider briefly some of the conditions which modify their activity. I am aware, however, that I am dealing with a much abused subject, one possibly worn threadbare, certainly one, concerning which, of late years, much has been said and written. But there are many conflicting statements, many downright contradictions, and I have ventured out of my experience in the laboratory to present to you the results of my own observations, enlarged somewhat by those of others which have seemed to me worthy of credence. A somewhat continuous study of the digestive ferments in their relation to normal digestive action has led to an accumulation of data (See Studies from the Laboratory of Physiological Chemistry. Sheffield Scientific School of Yale University, Vols. I-III), most of which, to be sure, has come from bottle and test-tube study, but yet I think is not to be ignored on that ground, nor on the other hand to be accepted necessarily in its entirety, but to be looked upon as a statement of fact so far as it goes, and as a suggestion to be tested clinically when of sufficient importance. So far as the pure chemistry of digestion is concerned, the nature of the ferments and their action, the influence of various agents on their activity etc., the laboratory is the proper place for such study and the data so obtained may be of great advantage in pointing the way for clinical experiments. It is to be ever borne in mind, however, that the living alimentary tract is a somewhat different mechanism from a glass beaker, and that in the former we have to deal with a complication of conditions not met with in our artificial digestions.

In considering first the action of the amylolytic ferments, we will speak only of the ptyalin of saliva and the diastase of malt, the one as an illustration of a normal digestive ferment, the other as a good example of a common remedial agent.

What is true, however, of the ptyalin of saliva is also applicable to the amylolytic ferment of the pancreatic fluid.

Human mixed saliva as ordinarily secreted has an alkaline reaction, the average of 51 samples showing an alkalinity equal to 0.08 per cent. sodium carbonate. The highest amount found was 0.144 per cent. the lowest 0.059 per cent. In spite of this being the normal reaction of the secretion, its power of digesting starch is far greater when the fluid is exactly neutral than when alkaline, a difference which shows still more distinctly the greater the fluid is diluted. The ferment acts most energetically in a neutral fluid. The same is true of the diastase of malt, its diastatic action showing stronger in a neutral fluid than in an

alkaline medium. Increasing the alkalinity of the fluid, either diastase or saliva, tends to retard the amylolytic action of the ferment, the extent of retardation being in proportion to the amount of alkali carbonate present. The percentage of alkali, however, which hinders diastatic action can be designated only for definite mixtures, being dependent upon the dilution of the fluid, and consequently upon the amount of albuminous matters and inorganic salts present.

The presence of 0.3—0.5 per cent. sodium carbonate will almost entirely stop the action of undiluted saliva on starch, while with neutral saliva greatly diluted, the presence of even 0.005 per cent. sodium carbonate will diminish decidedly the action of the ferment. Dilute alkalies not only hinder the action of these amylolytic ferments, but they also destroy them, especially at the body temperature. Their destructive power, however, is not as great as their retarding action. While these facts plainly indicate the extreme sensitiveness of the ferments towards alkaline fluids, we must not be too hasty in assuming a destructive action whenever alkalinity becomes pronounced. Peptones and proteid matters in general all tend to diminish and even prevent in part the retarding and destructive action of dilute alkalies, hence in the intestinal canal and elsewhere where the products of proteolytic action or other forms of proteid matter are present, the amylolytic ferments may endure the presence of amounts of alkalies which alone would quickly lead to their destruction.

Towards ac ds, the amylolytic ferments, both ptyalin and diastase are more sensitive even than towards alkalies. When diluted neutral saliva, or a solution of diastase, is mixed with diluted hydrochloric acid in such proportion that the mixture contains only 0.003 per cent. of the free mineral acid, amylolytic action is stopped almost completely. With 0.005 per cent. of free hydro-chloric acid., destruction of the ferment is complete in a very short time, especially at the body temperature.

It has been generally held hitherto, and is even now to some extent, that the ferment of saliva and diastase as well, regain their power of transforming starch into sugar when they reach the small intestines, where the contents are alkaline, this view assuming that in the stomach the activity of these ferments is simply suspended by the acidity of the gastric juice. It has even been questioned whether the acidity of the stomach contents ever becomes sufficiently great to completely stop the solvent action of the amylolytic ferments on starch. Many eminent authorities stand committed to this view of non-destruction by the gastric juice, but it is a question easily settled by experiment and I am quite convinced that the presence of a few thousandths of one per cent. of *free* hydrochloric acid is sufficient to quickly stop all amylolytic action. We are to bear in mind, however that because a fluid reacts acid, to test papers, it does not necessarily follow that it contains free acid. In gastric juice for example, especially after digestion is well under way, there are present comparatively large amounts of albuminoses, peptones, etc., all of which unite with the acid of the gastric juice, forming a *loose* chemical combination to be sure, but yet one in which the acid is far less powerful towards ferments at least, then when uncombined. Hence the question of retardation and destruction of amylolytic ferments in the stomach needs further consideration; we need to know how the proteid matter affects the action of the acid of the gastric juice and we find by experiment that nearly all forms of albuminous matter prevent to a certain extent the destructive action of the acid. The acid-proteids formed, however, have more or less of a destructive action themselves and when all the proteid matter present in a given mixture is completely saturated with acid, although no free acid may be present, the amylolytic ferments soon lose their action on starch, and in a short time are completely destroyed. Hence, it follows, that while the proteids of the food probably protect for a time the ptyalin of the saliva, or other amylolytic ferments introduced, by combining with the hydrochloric acid as it is secreted, in a very short time these must become saturated and free acid be

present, and as soon as free hydrochloric acid is present, or even before, a rapid destruction of the amylolytic ferments must take place. And to this destructive action must be added also the slower action of the acid-proteids. That free acid is normally present in the stomach contents can be easily shown by several tests, notably with tropæolin oo. The length of time after the ingestion of food, before free acid makes its appearance in the stomach, must be variable, dependent in great part upon the amount and character of the food taken. There is, I think, among many physiologists a growing impression that for 15 to 30 minutes after taking food an active digestion of starch goes on in the stomach. Von Velden* found, by methods perhaps somewhat questionable, that for a time varying from three-quarters to two hours after eating, the fluid in the stomach, obtained by a stomach pump, gave no color reaction with methyl aniline violet or tropæolin for free acid, although the mixture showed an acid reaction to test papers. Uffelman† likewise found a similar absence of free hydrochloric acid in the case of a boy with a gastric fistula and fed on a mixed diet, free acid appearing from forty-five to sixty minutes after the ingestion of food Kretschy and Seemann obtained similar results. More recent experiments of Ewald,‡ however, appear to show that the time of appearance of free acid depends entirely on the food; thus, in one experiment, with a person where vomiting could be produced at will, a meal of 60 grams of wheat bread was followed by the appearance of free hydrochloric acid in the stomach contents in thirty minutes; with hard boiled eggs even after fifteen minutes. With a moderate meat diet (120 grams) free hydrochloric acid was detected only after 1½ hours. Further, Ewald and Boas§ by experiments on inmates of the "Stœdtische Frauen-Siechen-Anstalt," Berlin, have found that on feeding starch paste (200-300 c.c.) made from either potato or wheat starch, free hydrochloric acid appears in the stomach contents very quickly. The experiments were conducted on patients with sound stomachs, the stomach being empty and indeed rinsed out with water just prior to the experiment and the wash-water proved free from acid. In this way they found that the ingestion of the starch paste was followed in one case by the appearance of free hydrochloric acid in ten minutes, the fluid vomited containing 0.04 per cent. HCl, the acid increasing after 27 minutes to 0.28 per cent. HCl. In another experiment after the same order 0.13 per cent. HCl was found in the fluid ejected after fifteen minutes, while at the end of thirty minutes the acid had increased to 0.29 per cent. In no case was lactic acid found in the ejected matter.

These same investigators have also in part confirmed our statements regarding the action of acids on the amylolytic ferment of saliva by a series of interesting chemical experiments on patients in the Frauen-Siechen-Anstalt. By feeding a one per cent. starch paste solution, to which a definite amount of hydrochloric or other acid had been added, to patients whose stomachs had been previously rinsed with water, they found that the smallest percentage of hydrochloric acid which would hinder the formation of reducing substances was 0.066 per cent. the stomach contents being ejected or withdrawn 5 to 45 minutes after the ingestion of the starch. With some patients, however, the acid could be raised to 0.1 or even to 0.12 per cent., and still have a trace of reducing bodies found, the latter being presumably sugar. With lactic acid, the amount could be raised to between 0.1 and 0.2 per cent., and still have some starch converted. With butyric acid 0.2 per cent., allowed some conversion. It must be remembered, however, that these percentages are simply the percentages of acid in the starch mixture, introduced into the stomach, and not the percentage of acid in the stomach contents, where there would naturally

*Zeitschrift für physiologische Chemie, 3, p. 205.
†Jahresbericht der Thierchemie, 1880, p. 302.
‡Virchow's Archives, Vol. 101, p. 362.
§Virchow's Archives, Vol. 104, p. 272.

occur a dilution and partial neutralization from the inflow of alkaline saliva, counterbalanced perhaps by the secretion of acid gastric juice. The most important point in this connection, however, is the fact that such conversion of starch as does occur in the stomach under these circumstances takes place during the first five minutes, the amount of sugar found in the ejected fluid being the same at the end of five minutes as at twenty minutes; further, the amount formed is quite small, implying that the ferment is quickly stopped in its action by the acid present. Ewald also concludes that the presence of 0.077 per cent. of hydrochloric acid is sufficient under the above circumstances to completely destroy the ferment. Coupling these facts with those already mentioned, I think we can safely conclude that the action of the diastatic ferments can at the best continue only for a short time in the stomach, and that cessation of amylolytic action is quickly followed by destruction of the ferment, through the action of the free and combined hydrochloric acid.

Further, it is obvious that the administration of diastatic ferments, however active, by the mouth, with the intention of supplementing the pancreative digestion of starch in the small intestines, can be of little value since the ferment must inevitably be destroyed before reaching the seat of action.

The extreme sensitiveness of the amylolytic ferments towards acids is substantiated by their behavior towards many common therapeutic agents; for the quantitative data, showing the exact amount of retardation or stimulation of amylolytic action, see Vol. I of Studies in Physiological Chemistry, Yale University. Many of the so-called antiseptics and germicides likewise show marked action on these ferments even when present in very small quantities. Mercuric chloride or corrosive sublimate, also mercuric iodide and bromide retard the action of the amylolytic ferments, even when present in a few thousandths of one per cent. Curiously enough, mercuric cyanide, when present in small amounts, appears to increase the solvent action of these ferments on starch. Large percentages, however, retard their action. Sulphate of copper has a very marked inhibitory action, while lead acetate has a retarding action only when present to the extent of two or three per cent. Arsenious oxide and ammonium arseniate in small fractions of a per cent., both cause neutral saliva to convert a larger amount of starch into sugar than the saliva alone would do, while arsenic acid retards the action of the ferment. Tartar emetic in small amounts has a marked stimulating influence on the salivary ferment, but large amounts, as 5 per cent., very noticeably diminish the amount of sugar formed. Potassium chlorate in small quantities increases the amylolytic action of saliva, while the presence of even 5 per cent. of the salt has only a slight retarding effect. Sodium chloride likewise has a slight stimulating action and large percentages cause only a slight diminution in the amount of starch dissolved. Many of the alkaloidal salts cause the salivary ferment to form an increased amount of sugar, apparently through stimulation of the ferment, notably morphine sulphate, quinine sulphate, cinchonine, and cinchonidine sulphates, atropine sulphate and brucine sulphate. Strychnine sulphate, on the other hand, has a slight retarding action on the ferment. Antipyrin and antifebrin both have a slight inhibitory action on the salivary ptyalin. Urethan, in small fractions of a per cent. has a slight stimulating action, while larger amounts diminish somewhat the quantity of sugar formed. Thallin sulphate in very small percentages has a noticeable stimulating action, while paraldehyde has a marked inhibitory effect.

Of gases, oxygen and carbonic acid both decidedly increase the amount of sugar formed by neutral saliva, while hydrogen noticeably diminishes the action of the ferment.

Pepsin, the best known of the proteolytic ferments, and perhaps the most important, has been the subject of study for many years. Ever since Eberle in 1834 called attention to the solvent power of an acid extract of the stomach mucosa, investigators have been at work in a vain attempt to isolate the active principle in a pure state. Schwan named the hypothetical substance, pepsin, and Wasmann just fifty years ago made an elaborate but fruitless attempt to isolate the pure ferment. Even at that time the power-

ful digestive properties of the ferment were recognized, for, Wasmann states that a weak acid solution containing only $\frac{1}{40000}$th part of the impure ferment will dissolve coagulated albumen in from six to eight hours. A long row of illustrious names may be added to the list of those who have endeavored to widen our knowledge of this proteolytic ferment; Pappenheim, Valentin, Elsässer, Frerichs, C. Schmidt, and many others may be mentioned as among the first to work upon this subject, while nearly every prominent physiologist since has made some contribution to broaden our knowledge of this digestive ferment.

Among the many facts connected with the proteolytic action of pepsin which it is important for us to remember is that the acidity of the gastric juice is mainly due to free hydrochloric acid. The elaborate experiments of Bidder and Schmidt still stand the test of criticism and while we have many times, especially in disordered conditions of the stomach, lactic, butyric, acetic and possibly other acids present in the stomach contents, we are to look upon them as the products of various forms of fermentation, rather than as secretory products from the stomach cells.

Richet (Du suc gastrique chez l'homme et les animaux) has claimed that the hydrochloric acid of the gastric juice does not exist free, but in a state of loose combination with leucin, as chloride of leucin. His experiments are of value, since they furnish added proof that the gastric juice contains but one mineral acid, but few physiologists are inclined to believe that it exists combined with leucin. Certainly for a vigorous gastric digestion, free acid is as indispensable as pepsin itself. Leucin is undoubtedly often present in natural gastric juice and in extracts from the stomach mucosa, but I have many times also found considerable quantities of xanthin, hypoxanthin and other similar crystalline extractives, and I see no reason for assuming a combination in the one case any more than in the other. As to the strength of hydrochloric acid in the gastric juice, Richet, as the mean of seventy observations on a patient who had had gastrotomy performed for an impermeable stricture of the œsophagus, found 1.3-1.7 per mille. Other physiologists give somewhat higher results and 0.2 per cent. is usually taken as the average content of acid in active gastric juice. It can be easily shown, however, by experiment that the strength of acid best fitted for digestion depends somewhat upon the amount of ferment present and the character of the proteid to be digested. Using a pepsin extract of moderate strength and blood-fibrin as the proteid to be digested, we have found by quantitative trials that the most vigorous proteolytic action is usually obtained in the presence of 0.1 per cent. pure HCl. Thus in one series of experiments where the amount of pepsin was the same throughout with 0.05 per cent. HCl 73.8 per cent. of the fibrin was dissolved; with 0.1 per cent., HCl 89.3 per cent. of the fibrin; with 0.2 per cent. HCl 84 per cent. of the fibrin; with 0.3 per cent. acid, 81.7 per cent.; while with 0.4 per cent. HCl only 63.8 per cent. of the fibrin was dissolved. It is also to be remembered that while the proteolytic action of the ferment is most vigorous in the presence of hydrochloric acid, other acids will to a greater or less extent take its place, viz., phosphoric, nitric, sulphuric, oxalic, acetic, lactic, etc. Thus with oxalic acid, proteolytic action is vigorous in the presence of 0.5-2.0 per cent. of the acid, most vigorous with 1.5 per cent such a mixture dissolving about three-fourths as much proteid as the same amount of pepsin with 0.1 per cent. hydrochloric acid.

With nitric acid, proteolytic action is most energetic in the presence of 0.2 per cent.; with sulphuric acid in the presence of 0.3 per cent. Compared with 0.1 per cent. hydrochloric acid, nitric acid is more than four-fifths as active, while sulphuric acid is little more than one-fourth as active and acetic acid is practically worthless. Hydrobromic and hydriodic acids can, to a certain extent, replace the hydrochloric acid of the gastric juice as Putzeys (Jahresbericht der Thierchemie, 1877, p. 279), has previously found, although they are both much less active than the latter. Moreover, hydrobromic acid is much more efficient than hydriodic acid in connection with the ferment, for in comparatively large doses the latter will completely stop all proteolytic action.

Whenever bromides and iodides are taken into the stomach they are supposed to be decomposed by the acid of the gastric juice with formation of hydrobromic and hydriodic acids respectively, by which the retarding action of these two salts on gastric digestion is produced. Hence, as a practical result the bromides and iodides should be given ½ to 1 hour before meals.

There are, I presume, many diseased conditions where imperfect digestion is due as much to the want of the necessary acid as to lack of ferment. Thus in fevers, as a rule, from whatever cause, a less active gastric juice is secreted than normal, one possessed of far less proteolytic action, though generally acid. The acidity, however, is frequently diminished and, as Ewald remarks, confirms the old habit of prescribing phosphoric or hydrochloric acid in fever mixtures. The simple fact that the stomach contents are acid does not necessarily indicate that the fluid is of the proper degree of acidity or even contains the proper acid, suited to the ferment. Acetic, lactic or butyric acid may be present and render the stomach juices decidedly acid and yet it may be necessary to give acid, in order to bring the acidity up to the point suitable for the best action of the pepsin. It is also possible to give an acid, as possibly salicylic, which will have a double action; viz., an antifermentative one and a digestion-promoting one. Certainly in many forms of dyspepsia, as the researches of Ewald have shown, the derangement originates in the absence of the required degree of acidity rather than in insufficiency of pepsin. In many such cases there may be an "acid stomach" and yet the secretion of normal gastric juice be practically suppressed, the acidity being due mainly to lactic acid doubtless formed by fermentation in the stomach; an acid which acts with pepsin only about $\frac{1}{3}$ or $\frac{1}{5}$ as well as hydrochloric acid. Occasionally, as you know, the stomach contents have an alkaline reaction, as when a strongly alkaline transudation is poured into the stomach in connection with diminished or entirely abolished secretion of acid. Again, there are many other forms of dyspepsia or gastric troubles where there is a relative insufficiency of secretion, where pepsin as well as acid is wanting and where artificial digestive preparations are especially called for.

With reference to the influence of drugs on the proteolytic action of pepsin-hydrochloric acid we have considerable definite information, partly as the result of experiments with artificial gastric juice and partly from observation on patients and animals with gastric fistula. Nearly all metallic salts diminish the proteolytic action of the ferment quite decidedly, even a few hundredths of a per cent., as a rule, producing a noticeable effect. Thus cupric sulphate, lead acetate, mercuric chloride or corrosive sublimate, mercuric bromide, iodide and cyanide, salts of tin, zinc, manganese and iron, all have more or less of a retarding action on the digestive power of pepsin. Iron salts retard the action of the ferment much more than the corresponding salts of manganese. Mercurous chloride or calomel has been shown by Wassilieff to have no action whatever on the ferment. The action of these metallic salts is due, as a rule, to the combination of the metal with the proteid to be digested, forming an indigestible compound, and in part to a direct action on the ferment itself. We have determined with all of these salts the exact amount of retardation or stimulation of peptic action under definite conditions, but I refrain from troubling you with the figures, especially as I think that the *extent* of action of a given amount of any drug in the stomach is, as a rule, greatly dependent upon the conditions, which are naturally variable, especially the strength of the pepsin-acid solution, the amount and character of the proteid to be digested, etc., and that it is better in applying these results to content ourselves with statements regarding the general nature of the action. Arsenious acid has a noticeable stimulating or accelerating action on the ferment, the presence of even 0.5 per cent. of this substance causing the pepsin mixture to dissolve a much larger amount of albumen than the pepsin-acid alone will do. Arsenic acid has the same action, only still more pronounced, and the presence of even 2 per cent. of this compound leads to increased proteolytic action. This certainly accords with the generally accepted views as to the influence of arsenic on nutrition in general.

Potassium permanganate has a very energetic action on pepsin, the presence of even 0.005 per cent. in a digestive mixture reducing the action of the ferment to one-quarter its normal. Potassium cyanide and ferrocyanide have marked inhibitory action on the ferment. Potassium chlorate and nitrate likewise retard the action of pepsin, and when present to the extent of 1.5 per cent. both salts reduce the proteolytic action to one-quarter that of the normal ferment. Sodium tetraborate or borax and the chlorides of sodium, potassium and ammonium, all retard the digestive power of the ferment. Sodium chloride in small amount, however, has a noticeable accelerating action. Potash and ammonia alum both retard digestive action. Sulphates of magnesia and soda likewise retard the action of pepsin, even 0.005 per cent., having a noticeable effect

Nearly all the alkaloidal salts have more or less of a retarding action on pepsin; thus strychnine, brucine, veratrine, morphine, narcotine, quinine, cinchonine and atropine sulphates all reduce the action of the ferment, morphine sulphate less than the others.

Bearing in mind that pepsin acts far less energetically with sulphuric and acetic acids than with nitric acid and with the latter less actively than with hydrochloric, we can easily see that, as a rule, everything else being equal, sulphates will retard the digestive action of pepsin more than nitrates, and the latter more than chlorides, and if we are to apply such results as these to our practice it would be to use chlorides of the alkaloids, where practicable, rather than sulphates, and the same of inorganic salts. To be sure, after a short time the alkaloid or its salt will have passed into the circulation and the stomach be freed from its influence, but it is well to heed the small things as well as the great, and if we can accomplish the same physiological effect with a chloride as with any other salt and thus avoid or lessen possible disturbance in the stomach it is perhaps as well to do so.

With alcohol we have a double effect to consider; the results of many experiments have shown plainly that the presence of alcohol impedes the proteolytic action of pepsin, even though it is present in comparatively small quantity, but as Gluzinski (Jahresbericht der Thierchemie, 1886, p. 263) has shown, alcohol rapidly disappears from the stomach, even 100 c. c. of 25 per cent. alcohol disappearing inside of 15 minutes. While in the stomach, alcohol undoubtedly retards the solution of proteid matter. Shütz finds that 2 per cent. has a retarding action, while 10 per cent. causes a very great retardation and 15 per cent. allows only a slight digestive action. Bikfalvi finds similar results as do likewise Ogata and Klikowicz. The disappearance of the alcohol, however, is followed by the secretion of an active, strongly acid gastric juice, which continues generally long after the food is entirely digested. Hence, under the influence of alcohol there is often an accumulation of large quantities of fluid in the stomach, frequently colored yellow by bile. With small quantities of alcohol, therefore, especially with an abundance of food, there is an undoubted stimulation of proteolytic action induced mainly, if not wholly, by the increased secretion of hydrochloric acid. Under such circumstances the first stage of retardation is hardly to be considered, since the alcohol disappears so rapidly. With large amounts of alcohol, the mechanical functions of the stomach are interfered with, and thus the food compelled to remain a much longer time in the stomach than normally.

Beer, wine and stronger spirits all have retarding action according to the experiments of Ogata (Archiv. f. Hygiene 3, p. 204) on a dog with gastric fistula. In the case of beer, Ogata found that the retarding action was due equally to the alcohol contained in it and to the extracted matters. Even sugar, both grape sugar and cane sugar, when taken in quantities above 10 grams, tend to retard the digestive action of pepsin, but on account of their rapid absorption such action is of course only temporary. Soda-water or carbonic acid water in quantities of 200 c. c. or more, moderately strong infusions of tea and coffee and 200 or 300 c. c. of spring water were all found to have no appreciable influence on gastric digestion in the stomach itself.

Sodium salicylate (Klikowicz, Jahresbericht der Thierchemie, 1885, p. 277) in doses of

from 2.5 to 5 grams has a marked retarding influence on the digestive action of pepsin. Chloral hydrate, according to Klikowicz, is without action on pepsin in doses up to 1 gram. With 2-3 grams, however, there is noticeable retardation of digestive action, which with larger doses becomes still more pronounced.

Among the newer drugs, antipyrin and antifebrin, both retard the action of pepsin; antipyrin, when present to the extent of 3 per cent., almost entirely stopping the action of the ferment. Paraldehyde has a very pronounced stimulating effect when present in small quantities, and even 2 per cent. has only a slight retarding effect. Urethan has a very slight inhibitory effect, while thallin tends to increase the digestive action of pepsin.

In contact with dilute sodium carbonate, pepsin is very quickly destroyed, especially at the body temperature. Experiments made with scale pepsin and pepsin extracts from the stomachs of various animals have shown plainly that destruction invariably takes place in the presence of 0.05 per cent. of the alkali carbonate, hence when the acidity of the gastric juice is neutralized in the small intestines and the mixture becomes alkaline, there will be a rapid destruction of the pepsin, aided, as Langley has found, by the trypsin of the pancreatic fluid.

Trypsin, the proteolytic ferment of the pancreatic juice acts freely only in neutral or alkaline fluids, slowly and imperfectly in feebly acid fluids. Thus in an experiment on fibrin a neutral solution of trypsin digested 77 per cent. of the proteid, while the same amount of ferment in the presence of 0.4 per cent. sodium carbonate digested 96 per cent. in the same length of time, and in the presence of 0.1 salicylic acid only 44 per cent. of the proteid; under ordinary circumstances the ferment appears to act most energetically in the presence of 0.5 per cent. sodium carbonate, but will act even in the presence of 5 per cent. of the alkali salt. In no case will a salicylic acid solution act as vigorously as a neutral solution of the ferment. It appears, however, that in the acid-reacting fluid the ferment simply acts more slowly and if time be given, will ultimately approach the action of the neutral fluid. In such cases, however, the salicylic acid is not free, but combined with the proteid matter; free acids, either mineral or organic, even a few thousandths of a per cent. completely stop the proteolytic action of trypsin and the addition of dilute hydrochloric acid to a neutral trypsin solution will prevent all proteolytic action, even before the proteid matter is completely saturated; after which the acid quickly causes the death of the ferment. A glycerin extract of the pancreas, for example, on being warmed at the body temperature with even 0.05 per cent. hydrochloric acid soon loses its proteolytic action, and, as Langley has shown, the presence of pepsin aids in the destruction. Hence it is obvious that pancreatic extracts or ferments given by mouth can be of no value whatever, since the proteolytic ferment at least will undoubtedly be destroyed in the stomach before reaching its normal sphere of action. It seems to me very desirable, however, to be able to use the pancreatic ferments as an aid to pancreatic digestion in the small intestines. The use of such preparations, however, even though fortified by doses of sodium carbonate or bi-carbonate can avail little, since destruction must inevitably follow their entrance into the stomach. I have seen, however, proclaimed somewhere a form of capsule insoluble in dilute acid, but soluble in alkaline fluids, which if truly possessed of such properties could be made an easy means of introducing both the amylolytic and proteolytic ferments into that portion of the alimentary tract where they are capable of performing their characteristic functions. Without some such method of protection, it is of course useless to administer trypsin by mouth with any hope of gain to the economy.

As you doubtless know, the action of trypsin is peculiar in that there is no swelling of the proteid matter as in the action of pepsin and acid, but the albuminous substance is eaten into, crumbles, falls apart and then dissolves. Further, the action of trypsin is peculiar in that it not only converts the albumen into peptone, but also de-

composes a portion of the latter with formation of leucin, tyrosin, and other products. With pancreatic digestion, the digestive function of the alimentary canal reaches its highest point, and so far as proteolytic action is concerned, trypsin is undoubtedly more highly organized than its neighbor pepsin; the changes produced by it are more pronounced and deep-seated.

I would be glad to give you some idea of the relative activity of pepsin and trypsin, as proteolytic ferments, but this I can hardly do with exactness. In normal digestion the two ferments work under such divergent conditions and the products of their action are so different that it would perhaps be hardly correct to measure their relative action by the amounts of albumen they are capable of dissolving. Again, so far as I am aware, attempts to obtain the pure ferments for pharmaceutical purposes have not as yet been as successful with trypsin as with pepsin. Looked at from the purely physiological standpoint, I am of the opinion from my own experiments with the two ferments, that pure trypsin will prove to be more energetic in its action than pepsin, but the manufacturing chemists have yet to make a trypsin preparation equal in action to many of the brands of pepsin now in the market.

As a solvent of pseudo-membranes, as in diphtheria and in croup, the digestive ferments are certainly destined to prove of considerable value. Both pepsin and trypsin are recommended, but from a partial study of the various digestive ferments at present obtainable, I am inclined to consider pepsin as the more efficacious. If a trypsin preparation could be obtained in strength equal to many of the preparations of pepsin I should be inclined to its use, for the reason that it acts best in an alkaline medium, that it will eat into and disintegrate the fibrinous membrane, rather than first cause it to swell up, that the alkaline secretions of the buccal and other glands will favor its action, that the alkaline fluids possible to introduce with the trypsin may have a slight solvent action in themselves on the diphtheritic membrane, and that the ferment will act after the excess of alkaline carbonate has disappeared.

These minor advantages, however, are, at present at least, far more than counterbalanced by the much greater activity of the ordinary pepsin preparations. Further, a large number of experiments to demonstrate the influence of various therapeutic agents on the proteolytic action of trypsin have shown me that as a rule this ferment is far more sensitive to the presence of foreign salts and drugs than pepsin is, and while this fact need not be considered here, yet it may influence us somewhat in favor of the latter ferment. Trypsin, however, is not much affected by the powerful oxidizing salt potassium chlorate, the presence of even 5 per cent. of this salt causing only a slight diminution in the solvent power of the ferment.

As we have seen, the solvent action of pepsin on proteid matter is most pronounced in the presence of 0.1-0.2 per cent. hydrochloric acid, but a thin solution of pepsin with this acid would very quickly rinse down when sprayed into the throat, for the dissolving of pseudo-membranes. Admixture of glycerin will in part prevent this and keep the ferment for a longer time in contact with the surfaces to be dissolved. Obviously, the operation of painting or spraying must be frequently repeated in order to keep the surface well moistened with the digestive fluid. Again, since pepsin will not act at all in a neutral or alkaline fluid, it is plainly better to have the digestive mixture at the outset contain at least 0 3-0.4 per cent. actual hydrochloric acid. This will in part provide somewhat for the natural dilution of the acid and also for the neutralizing action of the saliva and other fluids. Further, acid of this dilution is innocuous and is a not unpleasant and cleansing mouth-wash. So long as the fibrinous tissue can be kept acid the solvent power of the ferment will be exerted, and in this connection it is to be remembered that there exists a mutual attraction between the acid and the proteid matter of the membrane by which the acid will be retained longer than by perfectly inert matter. It is to be remembered, however, that such dilute acid has a ten-

dency to swell up proteid matter and we can conceive of cases where such application might be deemed inexpedient. The capability of pepsin for dissolving blood fibrin is very great, and at the body temperature its action is quite rapid, and hence one would expect that under suitable conditions the fibrinous portion of a diphtheritic membrane would be attacked with considerable rapidity.

The ferment solution, however, should, be carefully brought to the body temperature prior to its introduction and the ferment itself should be of the strongest kind, so as to favor immediate action. The widespread use of pepsin for this and other purposes has led to the manufacture of large numbers of preparations of this ferment, some of which at least are of doubtful quality. This fact has been impressed upon me many times in the laboratory, where for various physiological purposes commercial pepsin has been employed. Further, during the last six months I have made a comparative study of a number of the more prominent pepsins in the market, determining quantitatively their relative proteolytic action. The general use of pepsin as a remedial agent in gastric troubles may well make us solicitous as to the character and strength of the preparation at our disposal, but as a solvent for pseudo-membranes, where rapidity of action is of the utmost importance and the life of the patient hinges on the result we should be doubly sure of the character of the ferment employed. The methods at present suggested by the different pharmacopœias for testing the digestive strength of pepsins or pepsin solutions are somewhat variable, both in respect to the strength of acid employed and in the character and condition of the proteid matter to be dissolved, and it may also be questioned whether the standard adopted is sufficiently high.

Nearly all of the methods now in vogue, either for pharmaceutical or physiological purposes, are based upon the older methods of Bidder and Schmidt, Ebstein and Greutzner Gruenhagen (Lee Herman's, Handbuch der Physiologie Band 5, 2ter Theil., p. 75–77), and shorn of their details consist essentially in a determination of the amount of coagulated egg albumen or blood fibrin, which can be dissolved by the ferment in a given time, an excess of proteid matter being present, and the amount of albumen dissolved being taken as a measure of the proteolytic action.

Such a method does indeed show which mixture or pepsin has the stronger digestive action but does not give a very correct idea of the *relative* proteolytic power, for while the conditions in such an experiment or series of experiments appear to be the same in each case, they are in reality often very unlike. For as Thompson (The Druggist's Bulletin, Vol. 2, page, 261,) in a recent article on "comparative pepsin testing" has well said, the amount of albumen in each test may be the same and also the volume of the fluid and the amount of apparent ferment, and yet as soon as the digestion commences the weaker pepsins quickly have more surface of albumen or fibrin to work upon than the stronger and therefore show better than they should. Again all who are familiar with pepsin testing can easily see that the condition of the proteid matter to be acted upon becomes a very important factor in such a test whether blood fibrin or hard boiled egg, the fineness of its division, the completeness of its coagulation, the thoroughness with which it is kept suspended in the digestive fluid, all tend to exercise a very important influence on the final result and are necessarily a source of frequent error.

Still again, the strength of acid recommended by several of the pharmacopœias is such as to be at least suggestive of the formation of considerable acid albumen, by which the apparent strength of the ferment is correspondingly increased. To obviate these difficulties and, if possible, to insure more accurate results in pepsin testing, I have devised the following method, based upon the fact that fluid egg albumen is essentially of the same degree of digestibility as coagulated albumen (Wawrinski. Hermann's Handbuch der Physiologie, Band V., 2ter Theil, p. 83), and that the ability to form albumose and peptone is

possibly a more accurate measure of proteolytic action than the power of simply dissolving coagulated proteids.

The albumen solution is prepared after the manner recommended by Schutz (Zeitschrift für Physiologische Chemie, Band IX, p. 581). A quantity of the undiluted white of egg is freed from globulin by the addition of hydrochloric acid of specific gravity 1.12, 4.2 c. c. to 300 c. c. of albumen, shaken gently, and after standing some hours filtered. The fluid will then be found to have lost its viscosity and to be perfectly clear. The acid will likewise have neutralized the alkali carbonate present and converted the phosphates into acid salts. The solution, however, will not contain any free acid. 10 c. c. contain approximately one gram of dry albumen. The exact amount can be determined in a sample by coagulation. The solution can be kept for some days, and so used in a large number of experiments. The testing is conducted as follows: Ten or twenty c. c. of the albumen solution are measured out with a pipette and introduced into a suitable receptacle, a definite volume of the pepsin solution, say 50 c. c., previously prepared by dissolving a weighed amount of the pepsin (50–500 milligrams of the pepsin in 1 litre of the acid, according to its proteolytic power), in 0.2 per cent. hydrochloric is added, and enough more 0.2 per cent. acid to make the entire mixture 100 c. c. The fluids are then placed in a bath at 40° C. and allowed to remain there for five or six hours. (The conditions to be so arranged as not to have more than 50–60 per cent. of the albumen at the most converted into soluble products). No stirring is needed, no attention of any kind other than to keep the mixtures at the proper temperature, and there is no possible error from variations in the mechanical condition of the proteid. At the end of the allotted time, the mixtures are heated to boiling and the acid neutralized by addition of the equivalent amount of sodium carbonate, best in approximately one per cent. solution. The unaltered albumen as acid albumen is at once thrown down as a heavy flocculent precipitate, and while still hot it is collected at once on a dry, weighed filter, washed thoroughly with boiling water and dried at 110° C. From this is easily calculated the amount of albumen converted into soluble products under the conditions of the experiment from which in turn can be calculated the relative proteolytic action of the pepsins tested. The figures so obtained, if the conditions have been properly arranged, give a much closer approach to the true proteolytic power of a ferment that any similar method with solid proteids, but even this does not tell the whole truth. There is still felt the influence already mentioned of the relative excess of unchanged albumen in those digestions where the ferment action is weakest and hence after having used the above method as a preliminary test it is necessary to have recourse to a modification of the principle made use of by Brucke (Vorlesungen über Physiologie, p. 303), years ago, and recently recommended by Thompson, of using sufficient of each pepsin or pepsin solution to convert the *same percentage* of albumen into soluble products. In this way only, so far as I am aware, can the true proteolytic power of pepsin or pepsin extract be determined.

After these methods I have tested the following brands of pepsin, obtaining as a preliminary result the following figures expressive of their relative proteolytic action.

The "Pepsinum Purum in Lamellis" having the highest digestive power is taken as the standard (100):

	Preliminary test of Relative Proteolytic action.
1. Parke, Davis & Co.'s Pepsinum Purum in Lamellis	100
2. Fairchilds' Pepsin in Scale	73
3. Scheffers' dry Pepsin concentrated	70
4. Jensen's Crystal Pepsin	56
5. Ford's Pepsin in Scales	54
6. North's Pure Pepsin	36
7. Boudault's Pepsin	35
8. Royal Chem. Co.'s Pure Pepsin	27
9. Scheffer's Saccharated Pepsin	16
10. E. Merck's Pepsin Germ. Pur. Pulv	11
11. Lehn & Fink's Powdered Pure Pepsin	0

From these data, which are the average of many results, we might infer that Fairchild's pepsin, for example, contains three-fourths as much active ferment as the Pepsinum Purum of Parke, Davis & Co. and that Ford's and Jensen's pepsin contain approximately half as much true ferment as the Pepsinum Purum. Such a conclusion, however, would be fallacious and to obtain the true measure of proteolytic action we must proceed further and determine next the relative amounts of the different preparations needed to produce a like result in each case. After this method we find, for example, that it requires about twice as much of Fairchild's and Scheffer's Pepsin to form a given percentage of peptone as of the Pepsinum Purum, and that of Ford's and Jensen's preparations about three times as much, thus showing that the true difference in proteolytic power is considerably greater than the preliminary results alone indicate. As a final result then we may consider the true proteolytic power of the above ferments compared with the one of highest digestive power to be as follows:

	Relative Proteolytic Action.
1. Parke, Davis & Co.'s Pepsinum Purum in Lamellis	100
2. Fairchild's Pepsin in Scale	52
3. Scheffer's dry Pepsin, concentrated	48
4. Jensen's Crystal Pepsin	35
5. Ford's Pepsin in Scales	32
6. North's Pure Pepsin	16
7. Boudault's Pepsin	14
8. Royal Chem. Co.'s Pure Pepsin	9

In considering these results it is to be borne in mind that the same brand of pepsin is liable to slight variations in its digestive power, doubtless dependent in part upon the condition of the membranes from which it is prepared. Thus in many instances I have found one or two of nearly the same digestive strength changing their relative positions, notably, Nos. 2 and 3 and Nos. 4 and 5.

As to the actual strength of these preparations 1 milligram of the strongest pepsin converted into soluble products 198 milligramms of the pure dry albumen, which would be practically equal to 2000 parts of fluid egg-albumen.